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Opportunistic infections

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Researchers use viral replication to screen for modulators of ligand-gated ion channels.

Like the paintings hanging in hotel rooms, viruses are designed for reproduction. Viruses hijack infected cells to produce new viral particles. However, most research techniques and gene therapy strategies use replication-deficient viral vectors that allow the insertion of the viral genome into the host genome but prevent the production of new viral particles. Now Srinivasan *et al*. report a high-throughput screen in which the number of viral particles is a readout for ligand-gated ion channel modulation in a recent article in *Nature Methods*.

High-throughput screens have identified small-molecule modulators of ion channels. However, no existing methods screen for gene products that regulate ion channel function. Cellular proteins can modify the localization, sensitization and gating properties of some ion channels. For example, annexin II promotes the membrane localization of $Na_V1.8$ sodium channels, and in K_V4 potassium channels, heteromeric and homomeric ion channels have different properties.

Capsaicin, which makes chili peppers spicy, and noxious heat activate the transient receptor potential cation channel subfamily v, member 1 (TRPV1). The authors replaced immediate-early genes required for replication in the herpes simplex virus 1 (HSV-1) vector with *Trpv1* cDNA driven by the thymidine kinase promoter. They infected complementing cells that express the missing immediate-early genes, allowing viral replication. Capsaicin induced inward current in complementing cells expressing *Trpv1*, indicating that the viral vector produces functional ion channels.

Capsaicin increased intracellular calcium in cells expressing *Trpv1* but not an empty vector. Hyperosmotic shock induces mitochondrial permeability transition, which precedes both apoptotic and necrotic cell death. Capsaicin induced this process in cells expressing *Trpv1* but not an empty vector, suggesting that capsaicin induces calcium ion overload in cells with *Trpv1* expression driven by a strong promoter. Because capsaicin induced cell death, *Trpv1*-expressing cells showed reduced viral titer relative to cells infected with a control vector. Trpv1 antagonists, including ruthenium red and resiniferatoxin, blocked the capsaicin-induced reduction in viral titer, suggesting that virus production correlated with Trpv1 inhibition.

Poreless Trpv1 associates with wild-type Trpv1 subunits to form heteromeric channels that are insensitive to capsaicin and impermeable to calcium. In complementing cells infected with HSV-1 vectors containing both poreless and wild-type *Trpv1*, capsaicin treatment selected for cells expressing poreless *Trpv1*. Following

capsaicin treatment, viral output was nearly normal in cells expressing poreless *Trpv1* and almost nonexistent in cells expressing only wild-type *Trpv1*.

The authors' system can be used to test a variety of ion channels. They infected cells with HSV-1 vectors containing the anion channel human alpha-1 glycine receptor (*GlyR 1*) cDNA. Similar to capsaicin treatment in cells expressing Trpv1, glycine induced inward current and reduced viral titer in cells expressing *GlyR 1*. Both effects were blocked by the GlyR 1 antagonist strychnine.

Presumably, this technique could be used to screen cDNA libraries for negative modulators of ion channels. The authors also suggest that this method could be used to screen RNA interference libraries for genes important in ion channel function.

Debra Speert

1. Srinivasan, R. *et al.* An HSV vector system for selection of ligand-gated ion channel modulators. *Nature Methods* **4**, 733–739 (2007). | Article | PubMed | ChemPort |